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ESTIMATION OF SOME IONS AS A TOOL FOR DIFFERENTIATION BETWEEN ANTE- AND POSTMORTEM SKIN WOUNDS

(With 3 Tables and 9 Figures)

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قياس بعض الأيونات كوسيلة للتفريق بين الجروح الحيوية والغير حيوية

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هذه الدراسة أجريت لغرض التفريق بين الجروح الحيوية والغير حيوية في الجلد وذلك بقياس بعض الأيونات بها مثل النحاس والحديد والزنك والكالسيوم والماغنسيوم بعد إحداث جروح مُفتعله في جلد أرانب بلديه. وقد أشارت النتائج التي تم الحصول عليها إلى ما يأتي: **أولا:** في حالة الحيوانات التي تم إحداث جروح بها قبل النفوق ، عند مقارنة تركز الأيونات في الجلد المجروح قبل النفوق في كل أوقات أخذ العينات بتركيزها في وقت الصفر (بداية إحداث الجروح الحيوية). أظهرت النتائج: (١) عدم وجود تغير في مستوى أيونات النحاس. (٢) وجود إرتفاع معنوي في مستوى أيونات الحديد بدءاً من ٣ ساعات حتى ٤٨ ساعة. (٣) وجود نقص معنوي في مستوى أيونات الزنك عند ٤٨ ساعة فقط. (٤) وجود إنخفاض معنوي في كمية أيونات الكالسيوم عند ٦ ساعات ثم تراجع بالزيادة إلى مستواه كما في وقت الصفر متبوعا بنقص المستوى ثم الرجوع إلى المستوى العادي وبعدها أستمر النقص في المستوى من ٣٦ ساعة حتى ٤٨ ساعة. (٥) وجود انخفاض معنوي في أيونات الماغنسيوم بدءاً من ١٢ ساعة حتى ٤٨ ساعة. **ثانياً:** في حالة الحيوانات التي تم إحداث جروح بها قبل النفوق: عند مقارنة تركز الأيونات في الجلد السليم قبل النفوق في كل أوقات أخذ العينات بتركيزها في وقت الصفر (بداية إحداث الجروح الحيوية) تبين وجود إنخفاض معنوي في تركيزات كل الأيونات في كل الأوقات ماعدا الحديد الذى بدأ إنخفاضة من ١٢ حتى ٤٨ ساعه. **ثالثاً:** في حالة الحيوانات التي تم إحداث جروح بها بعد النفوق، في حالة الجروح التي أحدثت بعد الوفاة لم يوجد أى تغيير معنوي في تركيز أيونات الماغنسيوم عندما تم مقارنته بتركيزه في وقت الصفر (بداية إحداث الجروح الغيرحيوية) بينما لوحظ وجود إنخفاض معنوي فى تركيزات النحاس والحديد والزنك والكالسيوم في عينات كل من الجلد المجروح والجلد السليم. **ومن** النتائج التي تم الحصول عليها تبين لنا أن أيونات النحاس والحديد والزنك والكالسيوم والماغنسيوم يمكن استخدامها كمؤشر مفيد للتفريق بين الجروح

والجروح الغير حيوية وأن استخدامهم الروتيني المتكرر في مجال الطب الشرعي التطبيقـــي يحتاج إلى دراسات أخرى.

SUMMARY

The present study was carried out to estimate some ions for differentiation between ante-mortem and post-mortem skin wounds. Skin samples were taken from ante-mortem and post-mortem skin wounds of balady rabbits. The concentration of copper, iron, zinc, calcium and magnesium was determined. Our obtained results showed that: **FIRSTLY**, **[A]** In case of vital wound, the concentration of ions (copper, Cu; iron, Fe; zinc, Zn; calcium, Ca and magnesium, Mg) in wounded skin were as following when compared with the value of zero time of vital wound induction: **(1)** copper ions had no significant changes, **(2)** iron ions had significant increase in all intervals, **(3)** zinc ions only showed significant decrease only at 48 h, **(4)** calcium ions were significantly decreased at 6 h and from 36 h upto 48 h, and **(5)** in case of magnesium ions, they were significantly decreased from 12 h upto 48 h. **[B]** also in case of vital wound, the concentrations of ions (Cu, Zn, Ca and Mg) in intact skin were significantly decreased in all intervals when compared with the value of zero time of vital wound induction except the concentrations of Fe which showed significant decrease only from 12 h upto 48 h. **SECONDLY**, **[A]** in case of post-mortem wound, the concentration of Cu, Fe, Zn, and Ca in wounded skin were significantly decreased when compared with the concentration of zero time of post-mortem wound induction, while Mg concentration showed no significant changes. **[B]** Ions concentrations of intact skin in post-mortem wounded skin showed significant decrease in Cu, Fe and Ca concentration when compared with the value of zero time of post-mortem wound induction. Our results demonstrate that ions (Cu, Fe, Zn, Ca, and Mg) are serve as a diagnostic markers for differentiation ante-mortem from post-mortem wounds, and for determination of the age of the wound especially in forensic practice.

Key words: *Rabbit- skin- ions- iron- antemortem & postmortem wounds.*

INTRODUCTION

In veterinary forensic practice, it is important to estimate wound age or to distinguish ante-mortem from post-mortem wound. The use of

ions as markers for differential diagnosis between ante-mortem and post-mortem wounds has been studied in humans and pigs and appeared to be a technical improvement (Hernandez- Cueto et al., 1982; Njau et al., 1991; Chen et al., 1995). The application of biochemical (Berg, 1972; Borriello et al., 1984 and Raekallio and Makinen, 1970), and enzyme - histochemical markers (Linder, 1982 and Raekallio, 1970) in the differential diagnosis between vital and postmortem wounds has suggested a significant technical improvement that may be used by medicolegal practitioners in confused cases.

Hernandez-Cueto et al. (1982) found that the ions Ca, Mg, Cu and Zn are useful in detection of vital and postmortem wounds, which have the ability to diagnose vital inflicted only 5 minutes before the moment of death. Njau et al. (1991) studied the Mg, Ca and Zn fluctuations of skin-induced injuries in correlation with time induction, also he found that mean Mg, Ca and Zn concentrations vary by time elapse and the ratios of Ca/Zn, Ca/Mg and Mg/Zn versus time present a curve from which the time of injury induction can be calculated. Chen et al. (1991) reported that experimentally induced ante-mortem burns could be differentiated from post-mortem ones by determination of K^+/Na^+ ratio. Chen and Co-workers (1995) reported that the concentration of Fe in skin and muscle and K^+/Na^+ in muscle are useful markers for differentiating ante-mortem from post-mortem wounds.

Autolysis does not influence the K^+/Na^+ ratio in myocardium (Zugibe, 1966; McVie, 1970 and Rammer and Jansson, 1976). Girela et al. (1989) studied the postmortem stability of the ions Ca, Mg, Cu, Zn and Fe of intravital wound in pigs. Their results demonstrated that these ions conserve their diagnostic ability to differentiate vital from postmortem wounds, independently of putrefaction up to 48 h after death.

Concerning the lack of literature about the use of ions as markers in veterinary forensic practice to differentiate vital and post-mortem wounds and to help in determining the age of wound, the present work was carried out.

MATERIALS and METHODS

In this study, 20 male balady rabbits (11 months old, with 3kg average weight) were used. The animals were divided into two groups,

group A (n = 12) was used for induction of vital wounds in skin and group B (n = 8) which was used for induction of postmortem wounds in skin. Each animal in both groups was wounded 3 times (each wound 3 cm of length) in the back (gluteus areas) using a surgical scalpel. Skin samples from the injured areas at intervals 0, 3, 6, 9, 12, 24, 36 and 48 hours were taken for ante-mortem wounds, while that for post-mortem wounds were taken at 24 - 48 hours after induction of wound. The normal skin from the opposite sides was used as control.

From each wound (either vital or post-mortem), 5 mm wide skin samples were taken from wound edges with approximately weight of 5 grams from each sample. Fat was removed from the skin specimens. After being washed with deionized water, specimens (5 grams) were placed in a 50 ml Pyrex beaker and 10 ml of concentrated HNO_3 (conc. 65%, Merck) and 10 ml $HClO_3$ (conc. 65%, Merck) were added and digested. The contents were evaporated to 1 ml of residue which hence diluted to 50 ml (Agemain et al., 1980 and Parker et al., 1968). Iron, copper, zinc, calcium and magnesium were measured by atomic absorption spectrophotometer (Buck Model 210 VGP, USA).

The statistical analysis for the obtained results was carried out by using students "t" test (Snedecor and Cochran, 1974).

RESULTS

The mean concentrations of ions (copper, iron, zinc, calcium and magnesium) were recorded in table 1-3 and figures 1-9. In Table 1, for the vital wound, the concentration of ions (copper, Cu; iron, Fe; zinc, Zn; calcium, Ca and magnesium, Mg) in wounded skin were as following when compared with the value of zero time of vital wound induction: **(1)** copper ions have no significant changes, **(2)** iron ions has significant increase in all intervals, **(3)** zinc ions only showed significant decrease at 48 h, **(4)** calcium ions were significantly decreased at 6 h and from 36 h upto 48 h, and **(5)** in case of magnesium ions, they were significantly decreased from 12 h upto 48 h (Table 1 and Fig. 1-3). The concentrations of ions (Cu, Zn, Ca and Mg) in intact skin were significantly decreased in all intervals when compared with the value of zero time of vital wound induction except the concentration of Fe which showed decrease only from 12 h upto 48 h (Table 2 and Fig. 4-6). In case of post-mortem wound, the concentration of Cu, Fe, Zn, and Ca in

wounded skin were significantly decreased when compared with the concentration of zero time of post-mortem wound induction, while Mg concentration showed no significant changes. Also in Tbale 3, the concentrations of ions of intact skin in post-mortem wounded skin showed significant decrease in Cu, Fe and Ca concentration when compared with the value of zero time of post-mortem wound induction (Table 3 and Fig. 7-9).

DISCUSSION

Our results showed that the Concentrations of Cu, Fe, Zn, Ca, and Mg of ante-mortem wounded skin are significantly higher than those of their controls at the same time of intervals. In case of ante-mortem wounded skin, the conentrations of Zn, Ca and Mg ions were decreased when compared with their concentrations at zero time of wound induction except iron which showed an increase in all time of intervals. On the other hand, the concentrations of these ions in post-mortem wounded skin were significantly decreased when compared with their values at zero time of induction.

The significant elevation of Fe in ante-mortem wounded skin returned to release inflammatory mediators in injured tissues, which may cause the small arteries and capillaries to enlarge, speeding the blood circulation, leading to accumulation of blood cells with high iron containing haemoglobin in and around the injured area. So the iron content in the injured area increased. The above mentioned reaction can not happen in the postmortem injured tissues, but the Fe content of postmortem wound was showed significantly decreased. This explain our results for elevation of iron in ante-mortem wound and its decrease in post-mortem wounds.

It has been observed that while there is decrease of Mg ions in serum; Mg increases concomitantly the recovering cells of the injured tissues, this means that Mg ions return to the injured tissues and help in wound healing. Furthermore, it plays a significant role in cell metabolism and enzyme regulation at injured sites. Magnesium alteration have been reported in both experimental animal studies and human beings after traumatic injuries and subsequent repair (Alrowaih et al., 1987). This is in contrast to our results where magnesium is decreased in ante-mortem wounded skin.

Concentrations of zinc were highly significantly elevated when compared with their controls. These results were supported by the fact that Zn shifts into injured tissue for repair (Van Rij et al., 1981; Tengrup et al., 1981). Trauma and surgical procedures result in a preferential mobilization of Zn into injured tissues (Loven et al., 1984). It has been noticed, however, that there is a transient decrease in plasma Zn level and increased excretion of it in urine as well as decreased Zn in erythrocytes and uptake following trauma (Van Rij et al., 1981). These previous results were differ from our results where Zn levels were decreased at 48 hrs in ante-mortem and in post-mortem wounds.

Crawford (1987) reported that Zn is an integral part of several metalloenzymes, takes part in numerous stages in nucleic acid metabolism and seems to be closely involved in the homeostasis of inflammatory cells as well as wound contraction, epithelialization and fibrosis.

A significant elevation of Ca concentration had been observed at the site of injury in ante-mortem wounded skin. That may attributed to the vital role of the coagulation processes. Both in burns and experimentally induced injuries, significant alterations of Ca were demonstrated. Hormones such as calcitonin (CT) and parathyroid are primarily engaged in homeostatic control of Ca and a prostaglandin which subsequently stimulates CT secretion to have Ca homeostasis controlled (Loven et al., 1984).

From the obtained results of ions concentrations (Ca, Mg, Fe, Cu and Zn), our results are in agreement with the previous studies (Hernandez-Cueto et al., 1982; Njau et al., 1991 and Girela et al., 1989). They reported that the assay of these ions was useful in detecting ante-mortem and post-mortem wounds.

The results of ante-mortem wounded skin revealed a significant increase in Fe (3 hrs upto 48 hrs), and a significant decrease in Zn (48 hrs), Ca (6, 12, 36 and 48 hrs), and Mg (12 hrs upto 48 hrs) while Cu level showed no changes. In case of post-mortem wounded skin, there is decrease in Cu, Fe, and Ca without any changes in Mg.

So, the decrease of Fe in post-mortem wounded skin in comparison with increase of it in ante-mortem wounded skin help as a diagnostic tool for determination the age of the wound as well as for differentiation between ante-mortem and post-mortem wounds.

In conclusion, it is apparent that the ions of Cu, Fe, Zn, Ca and Mg maintain their diagnostic ability to differentiate vital from postmortem wound upto the starting the putrefaction at 48 h old. Finally I conclude that the concentration of these ions are useful markers for differentiation of ante-mortem from post-mortem wounds, but their routine use in forensic practice needs further investigation.

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Table 1. Effect of injury induction (vital wound) on wounded skin ions concentrations (Cu, Fe, Zn, Ca, and Mg) in balady rabbits.

Ions	Control value at zero time of wound induction	Time post-vital wound induction (hours)						
		3	6	9	12	24	36	48
Cu (µg/g)	19.5 ± 1.690	17.5 ± 0.329	14.1 ± 1.471	15.6 ± 1.862	16.05 ± 1.407	17.11 ± 0.659	17.4 ± 1.363	17.5 ± 0.432
Fe (µg/g)	25.3 ± 1.569	54.1 ± 1.579**	50.4 ± 2.548**	49.3 ± 4.722*	48.1 ± 2.286**	39.3 ± 1.496*	38.2 ± 2.570*	37.1 ± 3.171*
Zn (µg/g)	21.1 ± 2.619	20.4 ± 1.276	18.9 ± 0.953	19.4 ± 1.062	20.5 ± 1.915	16.2 ± 0.903	14.7 ± 0.588	12.7 ± 0.758*
Ca (mg/g)	0.140 ± 0.004	0.125 ± 0.008	0.110 ± 0.007*	0.108 ± 0.013	0.107 ± 0.006*	0.134 ± 0.025	0.109 ± 0.003*	0.070 ± 0.007**
Mg (µg/g)	96.80 ± 2.882	94.10 ± 3.985	93.30 ± 1.033	86.40 ± 6.229	80.50 ± 2.948*	74.25 ± 3.596*	67.20 ± 7.567*	59.30 ± 5.105*

- Values were mean ± S.E.M.

*: Significantly different from control value (zero time value) at $p < 0.05$.

**: Significantly different from control value (zero time value) at $p < 0.001$.

Table 2. Effect of wound induction (vital wound) on intact (healthy) skin ions concentrations (Cu, Fe, Zn, Ca and Mg) in balady rabbits.

Ions	Control value at zero time of wound induction	Time post-vital wound induction (hours)						
		3	6	9	12	24	36	48
Cu (µg/g)	19.5 ± 1.690	10.61 ± 1.284*	6.70 ± 0.478**	4.79 ± 0.289**	2.93 ± 0.374**	3.11 ± 0.160**	2.68 ± 0.287**	2.41 ± 0.261**
Fe (µg/g)	25.3 ± 1.569	24.41 ± 1.207	24.27 ± 1.167	21.75 ± 1.622	19.13 ± 0.171*	10.20 ± 0.669**	14.26 ± 0.702*	16.87 ± 0.631*
Zn (µg/g)	21.1 ± 2.619	10.20 ± 0.294*	11.30 ± 0.464*	8.90 ± 0.778*	5.00 ± 0.309*	2.90 ± 0.249**	2.50 ± 0.169**	2.20 ± 0.090**
Ca (mg/g)	0.140 ± 0.004	0.082 ± 0.005**	0.047 ± 0.005**	0.046 ± 0.006**	0.041 ± 0.004**	0.05 ± 0.001**	0.041 ± 0.004**	0.028 ± 0.004**
Mg (µg/g)	96.80 ± 2.882	52.4 ± 2.559**	42.8 ± 2.779**	46.8 ± 2.536**	48.1 ± 2.876**	44.3 ± 3.586**	25.2 ± 2.764**	16.1 ± 0.909**

- Values were mean ± S.E.M.
*: Significantly different from control value (zero time value) at $p < 0.05$.
**: Significantly different from control value (zero time value) at $p < 0.001$.

Table 3. Effect of wound induction (post-mortem wound) on wounded skin and intact skin ions (Cu, Fe, Zn, Ca and Mg) concentrations in balady rabbits.

Ions	Control value at zero time of post-mortem wound induction	Values of ions in post-mortem wounded skin	Values of ions in post-mortem intact skin
Cu (µg/g)	13.900 ± 1.122	1.737 ± 0.152**	1.346 ± 0.112**
Fe (µg/g)	24.540 ± 1.238	15.913 ± 1.228**	12.721 ± 1.259**
Zn (µg/g)	9.500 ± 0.543	12.277 ± 0.970*	10.502 ± 0.699
Ca (mg/g)	0.105 ± 0.008	00.083 ± 0.003*	00.076 ± 0.003*
Mg (µg/g)	60.100 ± 3.447	60.325 ± 3.099	67.260 ± 3.340

- Values are mean ± S.E.M.

*: Significantly different from control value (zero time value) at $p < 0.05$.

**: Significantly different from control value (zero time value) at $p < 0.001$.

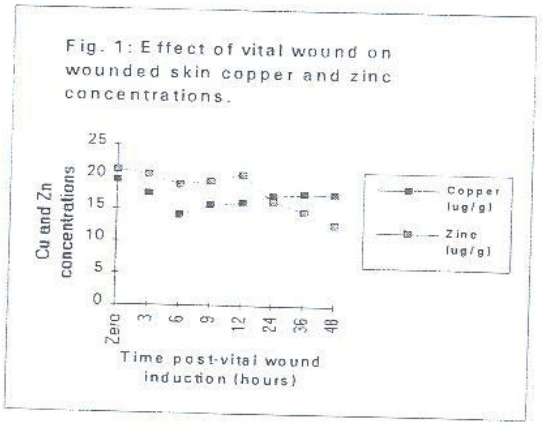

Fig. 2: Effect of vital wound on wounded skin iron and magnesium concentrations.

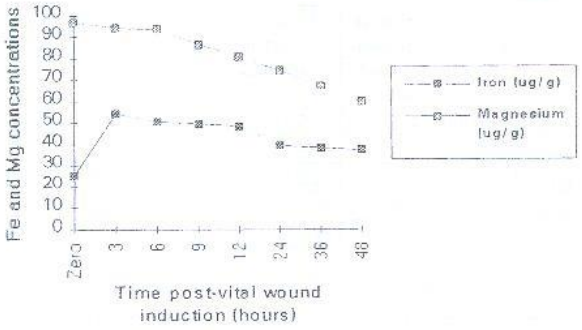

Fig. 3: Effect of vital wound on wounded skin calcium concentrations.

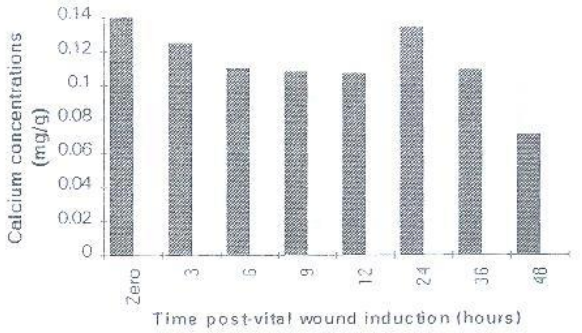

Fig. 4: Effect of vital wound on intact (healthy) skin copper and zinc concentrations.

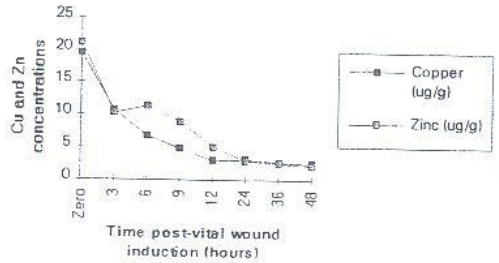

Fig. 5: Effect of vital wound on intact (healthy) skin iron and magnesium concentrations.

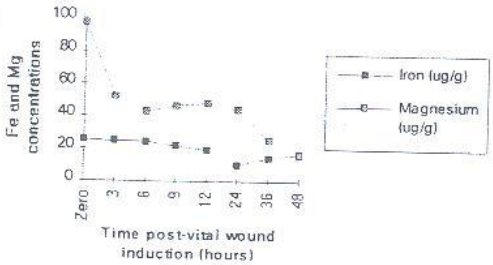

Fig. 6: Effect of vital wound on intact (healthy) skin calcium concentrations.

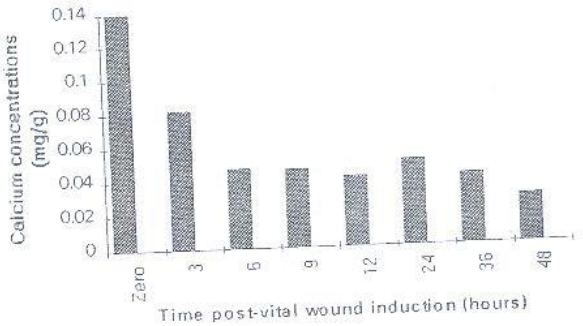

Fig. 7: Effect of post-mortem wound on wounded and intact skin copper and zinc concentrations.

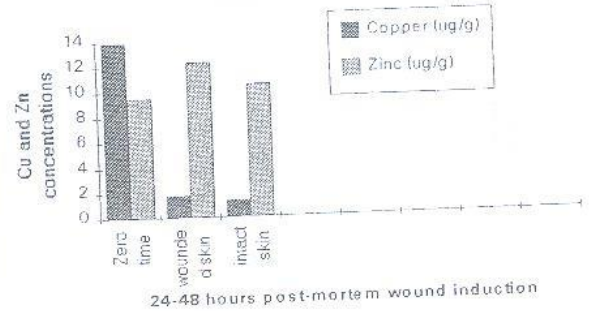

Fig. 8: Effect of post-mortem wound on wounded and intact skin iron and magnesium concentrations.

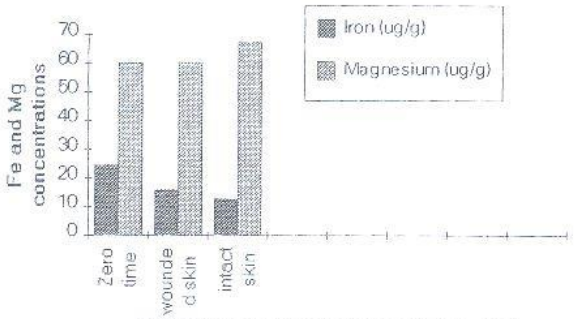

Fig. 9: Effect of post-mortem wound on wounded and intact skin calcium concentrations.